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Dying mRNA Tells a Story of Its Life

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In this issue of *Cell*, Pelechano et al. report that sequencing of mRNA decay intermediates shows surprisingly tight coupling of a major decay pathway to the movement of the last translating ribosome, revealing stress- and starvation-dependent modulation of translation elongation.

Messenger RNA lives an eventful life—each molecule is transcribed from DNA and then may be spliced, polyadenylated, modified, exported, transported, and translated before succumbing to degradation. Stages in the mRNA life cycle often overlap in important ways: splicing acts as a co-transcriptional quality control checkpoint (Chathoth et al., 2014), mRNA-ribosome complexes are cotranslationally localized to membranes by the signal recognition particle, and mRNA is degraded co-translationally (Hu et al., 2009). In this issue of *Cell*, Pelechano et al. (2015) show that 5′ → 3′ degradation of mRNA is tightly coupled to translation to the extent that sequencing mRNA during its destruction acts as a sensitive readout for the translational elongation movements of the last ribosome.

Mature mRNA has a 5′ cap, which breaks off, exposing hydroxylated (5′OH) ends, or is chewed away by the major 5′ → 3′ exonuclease Xrn1 and its kin, exposing 5′ monophosphorylated (5′P) ends. To quantify degradation at the genome scale, the authors developed methods to separately sequence each of these three classes of mRNA ends in budding yeast. The population of 5′OH turned out to be negligible, but 5′P mRNA sequencing—termed 5PSeq—yielded a substantial population, about one-eighth the size of the capped RNA pool.

The true surprise, the authors report, is that the positions of the ends of these 5′P molecules are not random in coding sequences but show pronounced three nucleotide periodicity, mirroring the codon-wise movement of ribosomes. This three nucleotide pattern depends on Xrn1 and is weakest near the start codon, which is consistent with the exonuclease chasing after, catching, and then closely following the last translating ribosome as soon as the cap is removed (Figure 1). 5PSeq detects codon-specific ribosome pausing in response to oxidative stress, amino acid starvation, and incorporation of amino acid analogs, thus passing key diagnostic tests for a measurement of translation elongation.

The apparent sensitivity of 5PSeq to elongation dynamics is important given recent controversy over estimates of elongation by the widely adopted method of ribosome profiling, which sequences RNA fragments protected by ribosomes from in vitro nuclease digestion. By contrast, 5PSeq sequences molecules resulting from in vivo degradation, omitting a major sample-processing step, yet appears to measure ribosomal dynamics at least as sensitively as ribosome profiling. The two assays in fact complement each other.

Neither 5PSeq nor ribosome profiling directly measures ribosomal movement, and so, although complementary, they each carry major caveats. In the case of 5PSeq, the necessity of removing the m^7GpppG cap to expose an Xrn1-chewable 5′P means that Xrn1 often idles while the last ribosome traverses the early 5′ mRNA region and then races to catch up. The resulting 5′P ends in this region—of unknown length—reflect a mixture of ribosome-mediated protection and cap-mediated protection. Which is which? How far does cap-mediated protection extend? How much do other mRNA features, such as base modifications and local structures, also impede exonuclease activity? Does the last translating ribosome move like all the preceding ribosomes, or do dynamics change during the lifetime of an mRNA?

Similar concerns have swirled around ribosome profiling, which has yielded some dramatic claims about ribosomal movement—prominently, the pausing of ribosomes at Shine-Dalgarno-like sequences in *E.coli* (Li et al., 2012) and around start codons in yeast but not at rare codons in either organism (Qian et al., 2012). The latter observation contradicts expectations from decades of research and has yet to be reproduced using other methods.

By contrast, 5PSeq detects pausing of the last ribosome at rare codons, but not around the start codon. Instead, Pelechano et al. (2015) show that the elongation inhibitor cycloheximide blunts the signals of sequence-specific pauses while causing ribosomes to pile up near the start codon. And, in a further contrast, 5PSeq detects an accumulation of ribosomes at stop codons that vanishes in cycloheximide-treated ribosome profiling experiments. More independent approaches are needed to distinguish the actual from the artifactual in genome-scale translation studies.

Recent related experimental work by Presnyak et al. (2015) shows that codon usage strongly modulates mRNA decay and protein levels, with some data to suggest that frequently used codons are translated more rapidly than rare synonyms. The codon-specific delays during mRNA destruction detectable by 5PSeq presumably relate to the elongation delays, which promote that destruction, but by what mechanism? One likely candidate is competition between translation initiation complexes and the decapping machinery. How might differences in ribosome elongation rate percolate back to initiation or decapping? Kinetic competition between ribosomes and RNA-binding factors that recruit decapping enzymes is one possibility; another is that slower ribosomal elongation could result in a lower

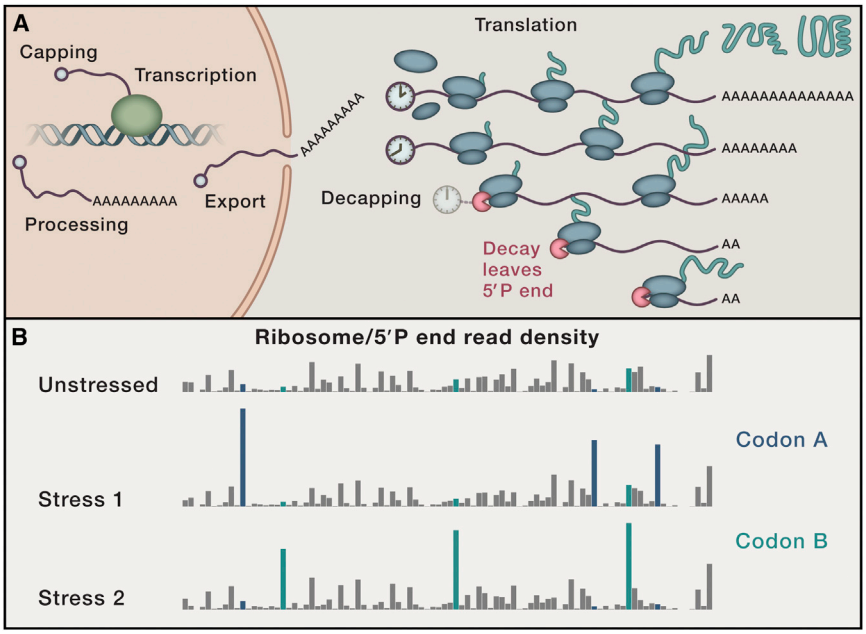

Figure 1. Messenger RNA Has a Complex Life Cycle
Life and death of an mRNA. After decapping, co-translational 5′ → 3′ decay by exonucleases (red) leaves 5′ ends, marking the last translating ribosome's position.

rate of initiation from recycled ribosomes reinitiating on circularized transcripts.

Mechanisms aside, these studies indicate that both the onset and the physical process of 5′ → 3′ decay, like other pathways of mRNA surveillance that occur on the ribosome (Shoemaker and Green, 2012), are coupled to translation.

In these genome-scale studies, we continue to see translation through a high-throughput glass, darkly. Debates rage about whether ribosomes pause at some codons, or amino acids, and not others, with seemingly minor differences in growth conditions, sample preparation, and statistical methods yielding incompatible results. Distinguishing biological phenomena from aberrations in the experimental glass remains challenging. What solid ground can the translation field stand on? Very strong signals pop out consistently, such as ribosome pausing at the SecM sequence in bacteria or codon-specific pausing during amino acid starvation (Subramaniam et al., 2014). Weaker signals may be detectable if amplified, such as by prolonging pausing by deletion of release factors (Guydosh and Green, 2014) or addition of artificial amino acid analogs. Details of RNA preparation, including inhibitors, 5′ chemistry, nuclease digestion conditions, and fragment length, may create or defeat artifacts and determine detectable phenomena. Which protocol details can be safely ignored? We do not yet know.

Substantial unexplained variation in read densities generated by high-throughput sequencing makes single-gene profiles difficult to interpret, and any individual peak or trough might be artifactual. Although statistical methods such as "metagene analysis" can reveal signals by aggregating across the transcriptome, any analysis pipeline might mislead and must be validated. It is unclear whether such methods are quantitative. Does a 2-fold increase in some model output correspond to a 2-fold decrease in ribosome elongation? In particular, failing to detect a signal with a particular high-throughput strategy (e.g., codon-specific pausing in ribosome profiling) does not mean the signal is absent. The signal may be detectable by alternative assays or even by alternative analyses of the same data. The arrival of 5PSeq provides valuable checks on the results of other high-throughput methods.

As the serendipitous discovery of sensitive last-ribosome dynamics exemplifies, the accumulation of new and independent methods continues to sharpen our global picture of translation in ways that will inspire future studies and confidence.

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